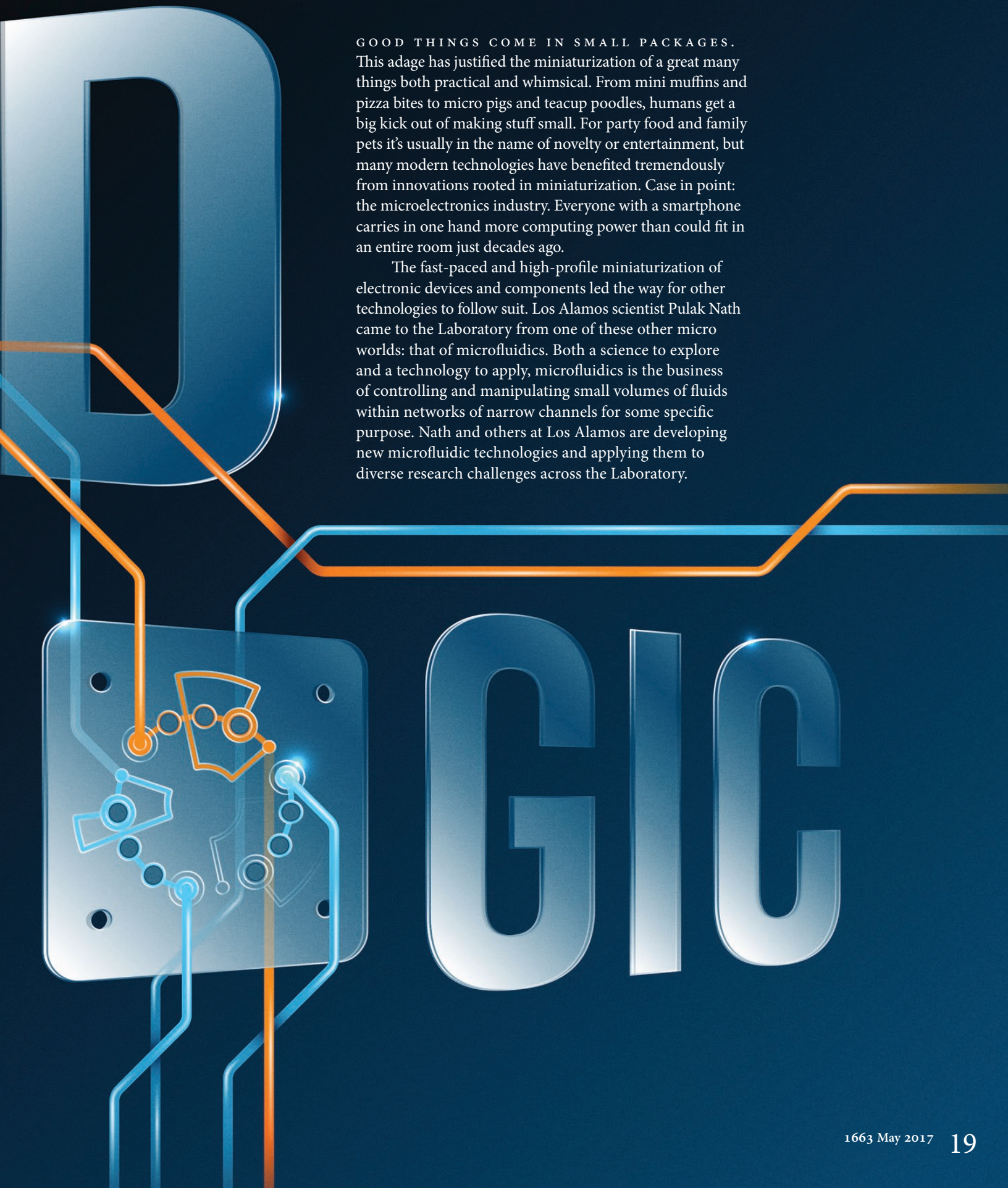
MICROFLUIDICS

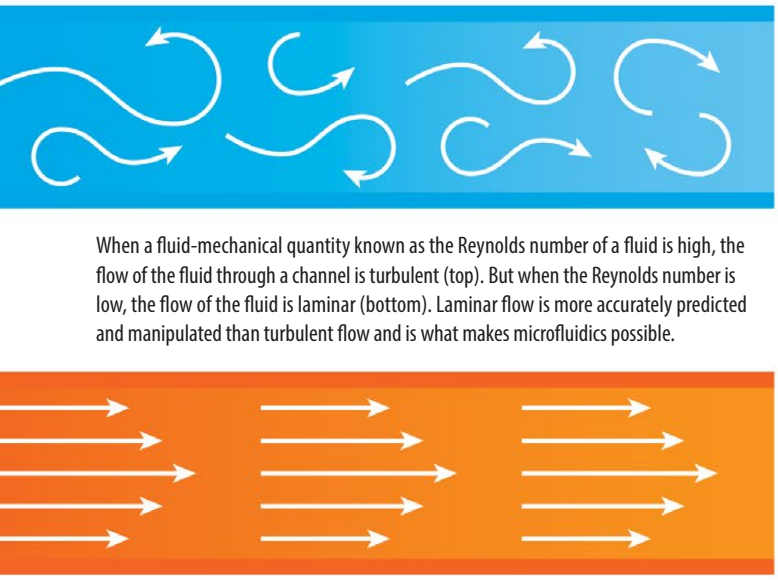
IS THE MANIPULATION OF LIQUIDS AT A VERY SMALL SCALE.

Los Alamos scientists are developing new ways to apply microfluidics to challenges in diverse fields from pharmacology to energy security.



GOOD THINGS COME IN SMALL PACKAGES. This adage has justified the miniaturization of a great many things both practical and whimsical. From mini muffins and pizza bites to micro pigs and teacup poodles, humans get a big kick out of making stuff small. For party food and family pets it's usually in the name of novelty or entertainment, but many modern technologies have benefited tremendously from innovations rooted in miniaturization. Case in point: the microelectronics industry. Everyone with a smartphone carries in one hand more computing power than could fit in an entire room just decades ago.

The fast-paced and high-profile miniaturization of electronic devices and components led the way for other technologies to follow suit. Los Alamos scientist Pulak Nath came to the Laboratory from one of these other micro worlds: that of microfluidics. Both a science to explore and a technology to apply, microfluidics is the business of controlling and manipulating small volumes of fluids within networks of narrow channels for some specific purpose. Nath and others at Los Alamos are developing new microfluidic technologies and applying them to diverse research challenges across the Laboratory.



When a fluid-mechanical quantity known as the Reynolds number of a fluid is high, the flow of the fluid through a channel is turbulent (top). But when the Reynolds number is low, the flow of the fluid is laminar (bottom). Laminar flow is more accurately predicted and manipulated than turbulent flow and is what makes microfluidics possible.

Laboratory protocols are essentially recipes that dictate a particular set of ingredients and order of steps—mixing, heating, cooling, separating—that yield a predictable product from raw materials. As Nath was ruminating on one of his protocols and how to make a microfluidic device for it, he decided he wasn't going to be able to do it with conventional methods. He needed a new way, and it had to be fast, affordable, and in-house for rapid prototyping. As he and his team honed their new manufacturing method, Nath realized that they weren't just solving the problem at hand, they were developing a unique platform which would enable whole new levels of microfluidic connectivity, integration, and automation. He named the platform "Liquid Logic."

"My daughter has these Lego sets that can be used to build different things from the same set of parts," Nath explains. "Maybe she builds a house one day and a car another day. Both are built from the same collection of pieces, but each uses a different subset from that collection. Our devices are intended to work the same way. They can be modular, so they can perform operations in whatever logical sequence a researcher needs. If one protocol says 'mix, heat, mix,' and another protocol says 'mix, heat, separate,' we can configure it either way with the modules we are building."

The devices are also intended to eventually adopt another, stricter version of "logic": if-then decision-making. For instance, a scientist could use a Liquid Logic chip connected to a camera to automatically sort microdroplets being used for a chemical reaction. If the protocol involves a color change indicator, such as "green means it worked, red means it didn't," then the chip can rapidly flow each droplet past a camera which will examine the color of each droplet. When the camera sees a green droplet go by, the chip will automatically open Valve A and shunt the droplet into a catchment reservoir. But when the camera sees a red droplet go by, the chip will close Valve A and open Valve B, sending the droplet into a waste receptacle. And a camera is just one example; the deciding variable could just as easily be pH, or temperature, or resistivity.

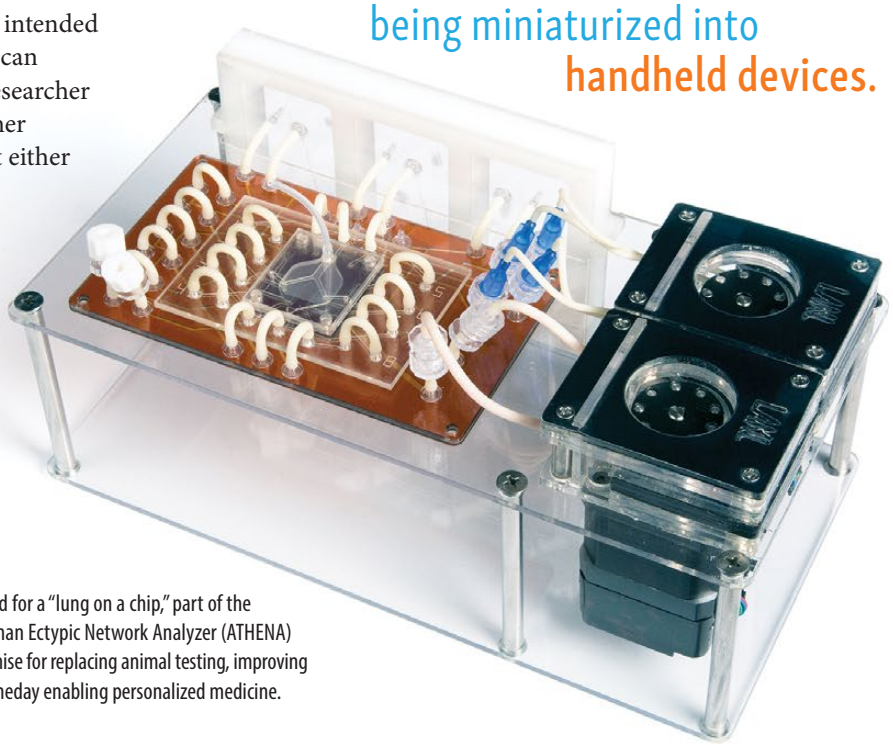
A big part of what makes the Liquid Logic platform unique is the manufacturing method that Nath and his team developed, which is a hybrid of additive and subtractive manufacturing. Sheets of various materials are precision cut with a laser (subtractive), to create channels, inlets, outlets, and even micropores in a membrane. Then the sheets are layered and laminated together (additive) to create the final device. Channels, valves, flow-pumps, vacuum-pumps, mixers, filters, and contactors can all be manufactured with the hybrid methodology.

Because many Los Alamos scientists use protocols that involve biological or radioactive contamination, the microfluidic devices they use have to be disposable. Typical microfabrication methods aren't often amenable to mass production, so once a proof-of-principle is achieved, a device may have to be completely re-engineered before it can be affordably mass produced. Not so with Nath's hybrid method—it relies on materials and equipment that are equally up to the tasks of same-day prototyping and affordable mass production and thus support disposable use applications.

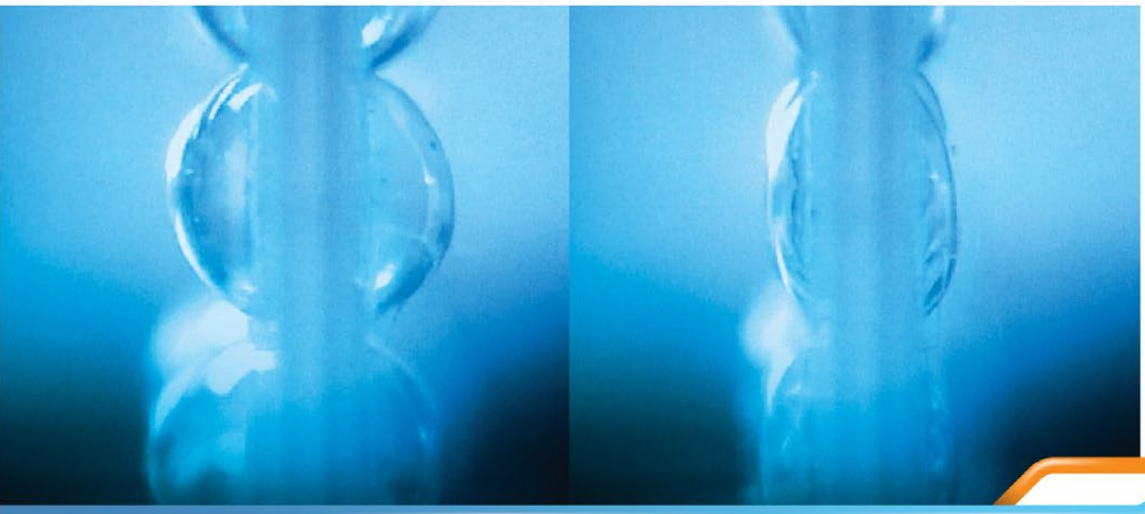
Hair-scale plumbing

Microfluidics is a rapidly evolving multidisciplinary field with applications in physics, chemistry, biology, biotechnology, nanotechnology, and engineering. Sometimes referred

Thanks to microfluidics, entire laboratories are now being miniaturized into handheld devices.



Fully automated fluid circuit board for a "lung on a chip," part of the Advanced Tissue-engineered Human Ectypic Network Analyzer (ATHENA) project. Artificial organs hold promise for replacing animal testing, improving toxicity screening, and maybe someday enabling personalized medicine.



Magnified and viewed from the side, the alveolar chambers of the Los Alamos bench-top model lung can be seen to breathe in (left) and out (right), just like a real lung. The human lung inflates when the diaphragm pulls down and creates negative pressure, which causes the lung to draw in air. With flexible membranes and a negative pressure pump engineered into the device, ATHENA's lung breathes the exact same way.

to as "hair-scale plumbing," microfluidics typically involves microliters of fluids (millionths of a liter) traveling through micrometer-scale (millionths of a meter) channels. An example of a familiar technology that relies on microfluidics is the inkjet printer, which quickly and precisely deposits minute amounts of liquid ink in prescribed intricate patterns.

The flow of a fluid through a microfluidic channel is described by its Reynolds number, which is calculated based on the fluid's viscosity, density, and velocity through the channel as well as the dimensions of the channel. Microfluidics permits low Reynolds numbers for liquids, which result in a smooth fluid flow known as laminar flow. The alternative to laminar flow is turbulent flow, in which the fluid moves chaotically, mixing and swirling like river rapids. But during laminar flow, the chaos clears and the fluid flows steadily along and becomes more precisely predicted, controlled, and manipulated. The flow is so calm, in fact, that multiple streams can flow simultaneously through the same channel, in the same or even *opposite* directions, with virtually no mixing other than basic diffusion.

The control of fluids within microfluidic devices is made possible by incorporating micro versions of the same types of mechanisms that handle larger volumes of fluids: pumps, valves, ducts, mixers, filters, separators, dispensers, sensors, and more. With clever engineering and design, microfluidic devices can perform complicated and elegant scientific maneuvers.

Lab on a chip

Just as a computer used to take up a whole room and now fits in our pocket thanks to microelectronics, entire laboratories are now being reduced to tabletop and handheld devices thanks to microfluidics. Chemical assays and biological systems that once required liters of reagents and days-long incubations are being redesigned to take advantage of microfluidics to save time, money, and space, with no cost to accuracy or sensitivity.

The Laboratory's Advanced Tissue-engineered Human Ectypic Network Analyzer (ATHENA) project is an example of lab-on-a-chip microfluidic orchestration. A collaboration between Nath and Los Alamos bioscientists Rashi Iyer and Jennifer Harris, among others, ATHENA is a project to develop artificial human organs that can emulate many of the functions of real human organs. Funded by the Defense Threat Reduction Agency, the team has, so far, developed a self-contained bench-top lung, heart (with Harvard University), and liver (with Charité – Universitätsmedizin Berlin, Germany). These three vital organs are key targets for toxins and infections, so it is paramount to have a reliable way of testing new potential remedies that circumvents the time, accuracy, and ethical challenges of conventional drug testing.

"Typically, to find out if a drug is toxic or if an antitoxin or antibiotic is effective, it gets tested first in petri dishes of cultured human cells, then in model animals such as mice and monkeys, then in human clinical trials," explains Harris. "But there's too big a gap between animal studies and clinical trials; we want to bridge that gap."

"The problem is that animals aren't humans, and cells in culture aren't organs," adds Nath. "Imagine having a transparent artificial organ system on a bench-top that exhibits the same biophysical and biochemical features as a human organ! We can see what is happening inside and probe it to learn new science that is not otherwise accessible."

But organs are incredibly complex—hearts beat and lungs breathe. The cells in our bodies are under constant surveillance and have systems in place to sweep away waste and deliver fresh resources. How can all of these functions be integrated and automated in a living, breathing, compact, bench-top device?

One key is to develop a fluid circuit board (FCB) to manage the device's intricate support systems. Just like an electronic circuit board, the FCB is intended to operate on a system of if-then logic gates to control the flow of fluids to and from the chip to provide nutrients and wash away waste, keeping the cells of the artificial organ alive for up to a month. For example, the production of mucus is required to recapitulate lung physiology. Healthy lung cells produce mucus to protect them against drying and inhaled

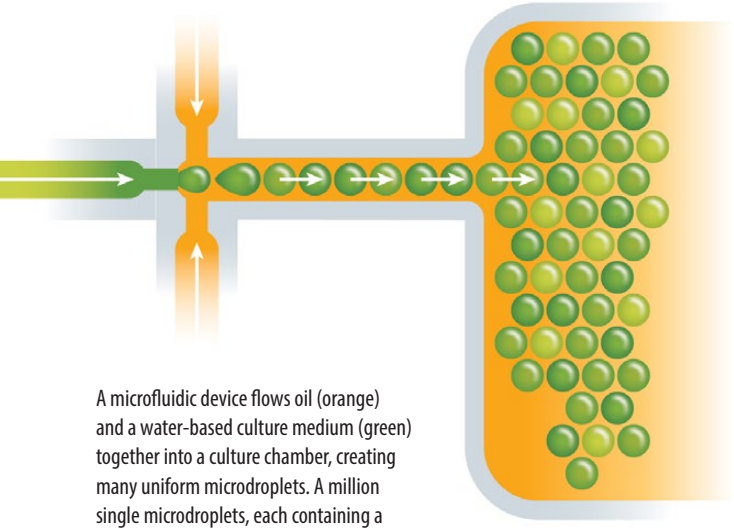
particulates. But if the mucus accumulates, it can cause the cells to die. So, the FCB can facilitate washing the cells once every 24 hours, keeping them hydrated, clean, and happy.

Another key to integration is the hybrid manufacturing method. The unique biomechanics of ATHENA's lung relies on this. Other artificial lung systems inflate the lung's alveoli by blowing them up like balloons, or by stretching them in one dimension like a rubber band, but that's not how a real lung works. A real lung inflates by negative pressure: the diaphragm pulls the lung open, forcing its alveoli to draw in air. By sandwiching flexible microporous membranes on either side of the fluid inlet within the lung device and then applying negative pressure with a microfluidic aspirator, the scientists were able to exactly replicate the mechanics of breathing. (The micropores are made with a special laser at the Lab's Center for Integrated Nanotechnologies; see "Femtosecond Fabrication," opposite page.)

Nath emphasizes the universality of the platform, saying, "ATHENA is an example of a marriage between engineering and biology, but with the Liquid Logic platform we are creating a suite of technologies that isn't limited to biology or engineering. It's for chemistry, it's for physics, it's for materials science. It is a completely unique capability that others at the Laboratory could benefit from."

Microdroplets by the millions

Some scientists around Los Alamos are indeed already taking advantage of it. Laboratory microbiologist Shawn Starkenburg studies different strains of algae as a source for biofuels in his energy-security work. An objective of this work is to learn which algae grow the fastest and best produce certain molecules that can be converted into biofuel. Many algae naturally grow in partnership with bacteria, so to get the best growth in the lab, Starkenburg has been testing different combinations of algae and bacteria. But there are so many different kinds of bacteria to test that finding the best pairing(s) requires screening millions of different combinations. Typically such screening would be done on 96-well plates measuring about 5 × 3.5 inches, with each well containing one combination. If Starkenburg wants to test a million combinations (which he does), he would need ten thousand plates, a lot of lab space, and tons of supporting equipment, reagents, and staff. And still he would need to work in batches, which takes more time and introduces more variables.



A microfluidic device flows oil (orange) and a water-based culture medium (green) together into a culture chamber, creating many uniform microdroplets. A million single microdroplets, each containing a unique combination of algae and bacteria, can fit in one handheld device, replacing at once 10,000 similarly sized culture plates.

> Microfluidics has been a **game-changer** for us in terms of **reducing radiation risk.**

Instead, Starkenburg and Nath, with others, are developing microfluidic platforms that can make quick work of such an intractable challenge. One device can create a million microdroplets that are all the same size (100–200 micrometers in diameter) and that essentially act as individual petri dishes for culturing algae and bacteria together. Each microdroplet of culture medium contains a tiny sphere of agar, a gelatinous medium used for growing microbes, that has been seeded with one algal cell plus 3–5 bacterial cells. Because all the microdroplets are contained on the same device, key conditions such as temperature and light are completely controlled for. After a week or two, the agar spheres are removed from their microdroplets and sorted by flow cytometry, which uses a laser to optically measure the sphere's chlorophyll content as an indicator of algal growth. The spheres with the best algal growth are sorted out, and their content is scaled up while the rest are discarded.

"We're at about a million microdroplets per device right now," says Starkenburg, "but we really want to scale to more than a billion. And there's no real reason we wouldn't be able to do that; the only limitation is the physical space of the culturing chamber." While the current, million-droplet culturing device contains a single monolayer of microdroplets, by transitioning to a deeper, more three-dimensional vessel like a flask or bioreactor, the billion-droplet goal ought to be easily reached.

Reducing the risk

Microfluidics is also providing big gains in nuclear-fuel-cycle facilities. The International Atomic Energy Agency (IAEA) requires, as part of its nuclear safeguards, strict accounting of all plutonium present in nuclear-energy facilities. Spent fuel, for example, has to be examined to determine how much plutonium remains. One of the IAEA mandates is that facilities be able to measure "spent fuel dissolver solution," a liquid into which some spent fuel has been dissolved. And to minimize the radiation hazard, the smaller the volume of such a liquid, the better.

A nice, fast, non-destructive elemental analysis technique for this purpose is x-ray fluorescence (XRF), which involves bombarding a sample with high-energy x-rays, then measuring the resulting emission of lower-energy x-rays to decipher the sample's elemental makeup. XRF is usually performed on

solid samples, but analysis of liquid samples is also important to IAEA fuel-cycle safeguards. The sensitivity of XRF when performed on a large volume of liquid isn't very good, but happily, it improves when a low-volume sample is used.

Los Alamos chemists Kate McIntosh and George Havrilla saw in microfluidics a perfect answer to the "how to do XRF on a small volume of liquid" challenge. They collaborated with Nath to design and build a microfluidic device that met the specific requirements for the task. It had to have spectral transparency that would allow x-rays to pass cleanly into and out of the device where the sample is contained, it had to be resistant to the strong acids into which the sample is dissolved, and it had to be cheap enough to make that it could be disposed of after one use. With Nath's hybrid manufacturing technique, prototypes were quickly whipped out and tested, and the design was refined accordingly. The result was a device that functioned as well as or better than a more expensive, commercially produced device. What's more, it is customizable, making integrated on-chip chemical separation possible in the future, thus further reducing the time and manpower it takes to get results.

"Microfluidics has been a game-changer for us in terms of reducing radiation risk while also improving data quality and workflow," says McIntosh. Nath envisions the Liquid Logic platform enabling a significant improvement to the state of the art, and McIntosh agrees. The next step is what she calls "at-line" analysis, wherein a processing facility will have an automated, on-site, microfluidic system making frequent, rapid, and accurate measurements rather than each sample going to an off-site lab that uses the old, large-volume, days-long technology. The microfluidic devices also save money since they are cheaper to make, cheaper to dispose of, and use far less radioactive material, thereby reducing those disposal costs as well.

The logo for the Laboratory's Center for Integrated Nanotechnologies (CINT), etched into the surface of a human hair. The hair, which measures approximately 0.1 millimeters in diameter, was both donated and machined by scientist Quinn McCulloch as a droll demonstration of the capabilities of CINT's custom femtosecond laser.

FEMTOSECOND FABRICATION

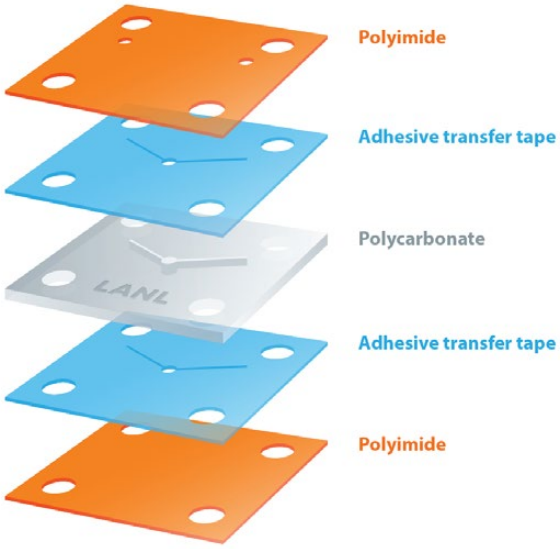
Traditional methods of creating microfluidic devices are usually advanced forms of lithography, a fabrication process that molds or etches features into thin layers of organic polymers. These methods are capable of creating microfluidic devices with incredibly fine and complex detail. But lithographic methods are limited in terms of the types of materials that can be used.

Science at Los Alamos often faces unique constraints that render conventional approaches or materials inadequate. For example, microfluidic devices may need to resist very strong chemicals or withstand high levels of radiation. Los Alamos scientist Pulak Nath was looking for a precision laser to help fabricate his microfluidic devices when he met Quinn McCulloch of the Laboratory's Center for Integrated Nanotechnologies (CINT). McCulloch is master and commander of a custom-built femtosecond laser that he uses to machine exotic materials well suited for extreme conditions. The laser can neatly etch microfluidic channels with features smaller than 10 micrometers (that's one one-thousandth of a centimeter, or roughly one tenth the width of a human hair). But the diminutive scale is only half of what's impressive about it. The other half is that the cuts are quite clean, especially compared to those made by more conventional, next-size-up nanosecond lasers. McCulloch's femtosecond laser produces cuts with no ragged edges or scarring to speak of, which otherwise could hinder the crucial flow patterns of fluids through the device.

The laser delivers ultrashort (less than a trillionth of a second) pulses of energy to its target—pulses so short that there is little-to-no thermal impact. Normally, laser cutting can burn the thing being cut, but a femtosecond laser is so fast that all of its energy goes to vaporizing material rather than melting or burning material at the edges of the cut. When it comes to micromachining exotic materials, McCulloch's laser is ideal.

Los Alamos earth scientists Hari Viswanathan and Bill Carey collaborate with McCulloch in their work studying geological fracture networks deep underground. Whether bringing material out of the ground (e.g., oil or gas) or putting material in to the ground (e.g., carbon dioxide or nuclear waste), fluid flow and transport occurring in subsurface fracture networks has to be well understood. Conditions at the surface aren't appropriate for modeling such phenomena—the temperature and pressure are too mild. Similarly, synthetic materials, though they may offer some advantages, are not as good as real rocks for understanding how real rocks behave. McCulloch makes microfluidic chips out of actual rock samples recovered from drilled cores, then Carey and Viswanathan load the chips with whatever fluid they're testing and place it into a special rig that applies heat and pressure comparable to 8000 feet below the earth's surface. Using real-time, high-resolution imaging, the scientists can evaluate where and to what extent the fluid travels through the microfluidic channels under such extreme conditions.

It's not practical to study fluid displacement in rocks 8000 feet below the surface of the earth. But with CINT's unique microfluidic capabilities, the exact conditions can be precisely replicated in the lab, bringing crucial light to an otherwise obscure natural system.



Cross-section diagram of a prototype device developed for conducting x-ray fluorescence spectroscopy, a method of elemental analysis, on small volumes of liquid spent nuclear fuel. The manufacturing method is lamination-based and uses layers of various materials.

Radioactive recycling

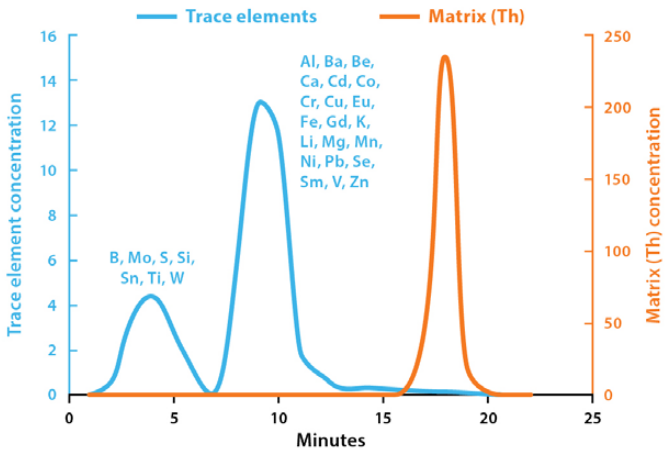
Laboratories that handle actinides like uranium and plutonium are strictly limited in terms of the overall quantity of material that can be present in the facility at any given time. These materials are termed "materials at risk," or MAR, and adherence to regulations, while paramount, also presents workflow challenges. Reducing the quantity of MAR needed for an experiment and the time needed to complete that experiment are therefore valuable advances that can streamline the workflow without compromising on safety. Los Alamos chemists Becky Chamberlin and Ning Xu have developed a way to reduce MAR quantity by up to 98 percent. And they did it with the help of microfluidics.

Plutonium is virtually nonexistent in nature, so it has to be manufactured, while uranium, though more abundant in nature, must be artificially enriched before it can be used industrially. During manufacture or recycling of these metals, it's important to know both the purity of the metal and the identity of any contaminating trace elements. The way this is usually done involves dissolving a small portion of the metal in a strong acid and then performing spectroscopy, which, in this case, identifies the elements in the sample by the wavelengths of light emitted when the atoms in the sample are excited by extreme heat. Unfortunately, plutonium emits light at a wide range of wavelengths, which can interfere with the identification and quantification of other elements in the sample. So the plutonium has to be separated out to allow the trace elements to be analyzed without that interference.

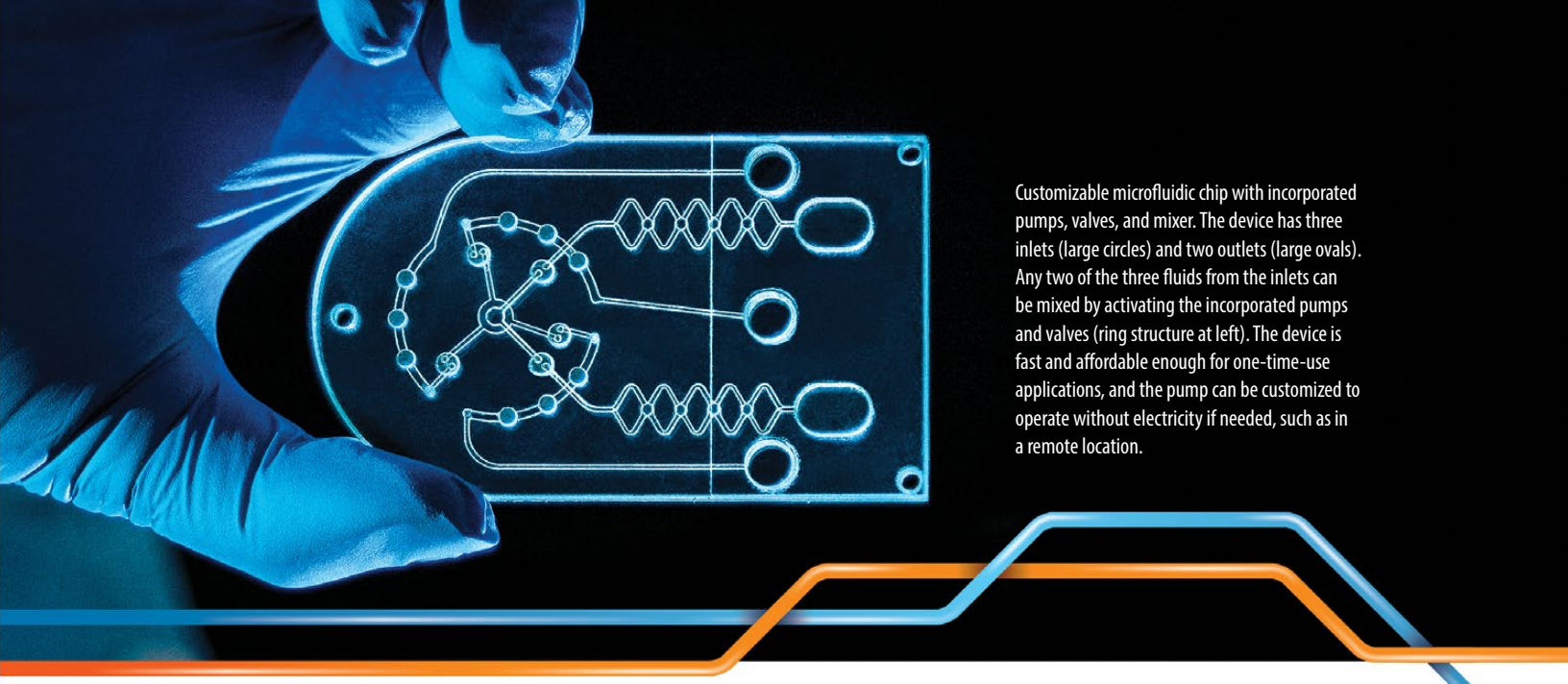
Chamberlin and Xu worked with Laboratory scientist Jun Gao to design a microfluidic device that efficiently separates plutonium from trace elements and which requires one-fiftieth the MAR quantity of traditional methods. The device incorporates resin microcolumns, through which the acid solution containing the dissolved metal sample is passed. The plutonium atoms bind to the resin and are retained in the column while the trace elements in the solution pass through. Then, upon subsequent washing, the plutonium atoms are released into a separate fraction. At this point, the trace elements and the plutonium can be independently analyzed. This type of analysis is also useful for determining the history of smuggled nuclear material or materials of unknown provenance—an important task for national security.

The microcolumn device uses a liquid-solid interface. But Chamberlin, with Los Alamos engineers Steve Yarbro and Quinn McCulloch, has also created a liquid-liquid system to study fundamental plutonium chemistry. Similar in concept to the microdroplet system used by Starkenburg in his algal biofuels research, this device creates many uniform microdroplets of an organic phase (oil-like liquid) surrounded by an aqueous phase (water-based liquid). The interface between the two phases, located at the surface of the microdroplets, is where important chemistry happens—molecules within the aqueous phase react with molecules within the organic phase. By using microdroplets, the ratio of interface surface-area to volume is greatly increased, bringing more reactants from the two phases into contact and making the interface chemistry more efficient.

The microdroplet device has also been engineered to separate the two phases again after they've reacted with each other. By coating the inside of the microchannels, their surface properties can be prescribed in such a way that one channel is highly hydrophobic (water repelling) and the other is highly hydrophilic (water attracting). So the team's device neatly separates the mixture, shunting the aqueous phase down one channel and the organic phase down the other channel. Here too, as with the trace-element separation, the two phases can now be examined separately to learn about the chemistry that occurred at the microdroplet interface.



Plot showing the total separation of a metal sample into thorium (orange, right scale), an actinide element used initially in lieu of plutonium, and small quantities of contaminating trace elements (blue, left scale). The metal sample is dissolved into a liquid and passed through a resin column integrated within a microfluidic device. As the sample travels through the column, thorium atoms bind strongly to the resin, while certain trace elements pass immediately through (left blue peak) and others take slightly longer to pass through (center blue peak). After the trace-element sample is collected, a wash with a different solution releases the thorium atoms from the resin column (right orange peak).



Customizable microfluidic chip with incorporated pumps, valves, and mixer. The device has three inlets (large circles) and two outlets (large ovals). Any two of the three fluids from the inlets can be mixed by activating the incorporated pumps and valves (ring structure at left). The device is fast and affordable enough for one-time-use applications, and the pump can be customized to operate without electricity if needed, such as in a remote location.

Onward flow!

Whereas logic in the general sense of sequential control systems is already guiding the design of Los Alamos microfluidic devices, logic in the strict sense is still coming along. But it's coming. The inclusion of automated if-then decision-making is the next phase for Nath and his collaborators. Integrating sensors that will measure temperature, color, or pH, for example, and take action based on those measurements, will bring Liquid Logic to the next level.

A lofty vision for the future, personalized medicine carries enormous potential.

Nath has lofty visions for the future of Liquid Logic and microfluidics across Los Alamos. One relatively new project involves the integration of microfluidics with a nuclear magnetic resonance (NMR) platform for chemical-threat detection. This collaboration will combine two bourgeoning Los Alamos capabilities: microfluidics and portable low-field NMR.

A goal for the immediate future is the further development of integrated functions. For example, McIntosh and Havrilla would like to include on their device the chemical separation steps of their spent nuclear-fuel measurement. This can be done with beads that selectively bind to certain ions, but it requires the addition of valves and pumps to orchestrate washing the beads. It also requires affordable mass production because the devices have to be disposable. Fortunately, once the engineering is done, the devices can be ultra-affordable, costing less than a dollar each when produced in large batches.

Another work in progress is the development of single-use devices with integrated features for applications in bioscience. The extraction of DNA or other molecules of biological interest from blood is a multistep process that can benefit tremendously from being automated on microfluidic devices. These will require much smaller volumes of sample and reagents, as well as less time to completion, and, as with radioactive applications, must be single-use. But no matter, as, like the devices for actinide analysis, they can also be mass-produced for a very comfortable price tag.

The loftiest vision for the future, though in no way unrealistic, is personalized medicine and the enormous potential therein. If Liquid Logic devices like the ATHENA lung were to be available in doctors' offices or hospitals, each and every patient could be diagnosed and treated with laser precision. The disposable device would house the patient's own lung cells—not mouse cells or monkey cells—living and breathing as lung cells do. Drugs could be tested in each patient's unique physiology before deciding which drug should enter the patient's actual body. No more guesswork and no more waiting games. That wouldn't just be a good thing in a small package, it would be the very best thing in a small package. LDRD

—Eleanor Hutterer

More on projects that use **microfluidics** at Los Alamos

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